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Clinical and microbiological features of bacteraemia with *Aerococcus urinae*

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Abstract

Aerococcus urinae is a Gram-positive bacterium that can cause invasive infection, including infectious endocarditis (IE), mainly in older men. *A. urinae* is often misclassified in routine diagnostic laboratories. Through searches in the laboratory databases we identify 16 isolates of *A. urinae* causing bacteraemia during a 6-year period in southern Sweden, indicating that bacteraemia with *A. urinae* occurs in at least three cases per million inhabitants per year. The identity of isolates was confirmed by sequencing of the 16S rRNA genes and antibiotic susceptibility testing identified two ciprofloxacin-resistant isolates. *A. urinae* was the only significant pathogen isolated in all cases. Fifteen of the 16 patients were male, 15/16 were more than 70 years old, and 12/16 had underlying urological conditions. Though a urinary tract focus was suspected in the majority of cases, the bacterium was rarely found in urinary samples. Nine patients fulfilled the criteria for severe sepsis and an additional four fulfilled the criteria for sepsis. Only one fatality was recorded. Patients were treated mainly with beta-lactam antibiotics but fluoroquinolones and clindamycin were also used. Three cases of IE were diagnosed and these were complicated by spondylodiscitis in one case and by septic embolization to the brain in one case. An increased awareness of *A. urinae* is crucial to establishing its role as an important pathogen in older men with urinary tract disease.

Keywords: *Aerococcus urinae*, bacteraemia, case series, infectious endocarditis, urinary tract infection
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Introduction

Aerococcus urinae is a Gram-positive coccus that was identified as a possible pathogen colonizing the human urinary tract and was originally designated as an *Aerococcus*-like organism [1,2]. The bacterium was recognized as a distinct species in 1992 [3]. *A. urinae* shares features with staphylococci, streptococci and enterococci as the bacterium grows in clusters, displays α-haemolysis when grown on blood agar, and is resistant to sulphonamide [1,2]. The organism is often misinterpreted as an α-haemolytic streptococcus in routine laboratory practice. Though correct identification of *A. urinae* with biochemical methods is possible, sequencing of the 16S rRNA gene remains the confirmatory test for identification [4]. The bacterium is generally susceptible to penicillin, vancomycin and cephalosporins, whereas it is resistant to sulphonamides [2,5]. Some reports have claimed that the organism is universally sensitive to ciprofloxacin [5], but recent studies indicate development of resistance to this group of antibiotics [4,6].

Aerococcus urinae is found in the urinary tract and most, but not all, studies implicate *A. urinae* as a pathogen in urinary tract infections [1,2,4,7,8]. Importantly, *A. urinae* has been described as a cause of invasive infections such as infectious endocarditis (IE) [9], spondylodiscitis [10], septicaemia [11], cellulitis [7] and peritonitis [12]. Patients who present with these types of severe infections are typically elderly men with underlying urinary tract abnormalities. Of 26

described cases of *A. urinae* IE, 21 patients were male and 20 were >70 years of age [11,13–18]. The case fatality in the described cases of *A. urinae* IE is above 50%, suggesting that IE with *A. urinae* is a very severe condition. *A. urinae* has the capacity to form biofilms and to aggregate human platelets, both of which could be important virulence mechanisms in IE [19]. The relatively low number of cases reported indicates that invasive infections with *A. urinae* are uncommon. Only two studies have attempted to define the incidence of such infections. In a nationwide survey in Denmark, Christensen *et al.* [11] estimated the incidence of bloodstream infections with *Aerococcus*-like organisms to be 0.5 cases per 1 000 000 inhabitants per year. In a Dutch report from a single laboratory, the incidence of bloodstream infections with *A. urinae* was estimated to be three cases per 1 000 000 inhabitants per year [15]. In this work we define the clinical presentation of invasive *A. urinae* infections in southern Sweden.

Methods

Isolates were from the two routine diagnostic Laboratories of Clinical Microbiology in Lund and Malmö, University and Regional Laboratories in Skåne, Sweden. The laboratories provide a service to ten hospitals, of which two are large university hospitals, in an area with a population of approximately one million inhabitants. Both laboratories employ the BacT/Alert blood culture system (bioMérieux, Marcy l'Etoile, France) with the FA/FN aerobic and anaerobic bottles supplemented with BHI solids and activated charcoal. Preliminary identification of bacteria from a positive blood culture relies on Gram-staining of the broth. If *A. urinae* is suspected from the appearance of the Gram-stain and growth on haematin agar, the laboratories precede with species determination with sequencing of the 16S rRNA gene (Lund) or Vitek 2 (Malmö; bioMérieux). Growth of an α-haemolytic bacteria in two or more blood cultures, demands a full species identification in both laboratories. For urine culture, 10 μL of urine was cultured on blood agar containing 5% horse blood for 16–20 h at 37°C with 5%CO_2. Species determination was carried out based on colony morphology and antibiotic susceptibility pattern.

The laboratories store all isolates from blood cultures at −80°C for 5 years, making reclassification of older isolates possible. Because *A. urinae* is easily mistaken for an α-haemolytic streptococcus, all isolates classified as 'α-haemolytic streptococcus' (Lund laboratory) or 'streptococcal species' (Malmö laboratory) between 2005 and 2010 were reviewed. Only isolates grown from more than one blood culture bottle were considered and all isolates classified as 'Gram-positive cocci in chains' on the initial Gram stain were disregarded. Of the remaining isolates, those with a zone for co-trimoxazole <12 mm were subjected to sequencing of the 16S rRNA gene. Sequence analysis of an approximately 900 base pair fragment of the 16S rRNA-gene was essentially performed as described previously [20]. The primers BAK11w (5′- AGA GTT TGA TCM TGG CTC AG -3′) and p91E-Revers (5′- CCC GTC AAT TCH TTT GAG T -3′) were used [21]. For determination of minimal inhibitory concentration (MIC), approximately ten colonies were suspended in saline to give a density of McFarland 0.5. Plates with Isosentitest agar (Oxoid, Basingstoke, UK) supplemented with 5% horse blood and NAD were inoculated with a cotton swab, and Etest strips were applied according to instructions from the manufacturer (AB Biodisk, Solna, Sweden). MIC values were recorded after incubation at 37°C in 5% CO_2 for 24 h or, if growth was not evident after 24 h, after 48 h.

Data on clinical presentation were gathered from the medical record of the respective patient. Organ dysfunction caused by the infection was assessed at the time of admission according to the guidelines of the Swedish Society of Infectious Diseases. Hypotension was defined as a systolic blood pressure <90 mmHg or a mean arterial pressure <70 mmHg; hypoperfusion was defined as a serum lactate >3.5 mM; respiratory dysfunction was defined as peripheral oxygen saturation <86%; renal dysfunction was defined as an increase in serum creatinine levels of >45 μM; haematological dysfunction was defined as a platelet count $<100 \times 10^9$/L or PK/INR >1.5; central nervous system dysfunction was defined as confusion; and hepatic dysfunction as serum bilirubin >70 μM. The local research ethical committee approved this study (2010/681).

Results and Discussion

A. urinae blood isolates

From the laboratory in Lund, ten patients with *A. urinae* in blood cultures from 2008–2010 were identified. The species identification had been made by sequencing of the 16S rRNA gene. In the Malmö laboratory, three patients with *A. urinae* in blood cultures had been recorded. The identification had been made by the Vitek 2 system (n = 2) and by sequencing of the 16S rRNA gene of one isolate. One of the isolates previously identified with the Vitek 2 system was identified as *Aerococcus sanguinicola* by 16S rDNA sequencing and thus disregarded. The other isolate identified by the Vitek 2 system had not survived freezing, making further analysis

impossible. Using the Vitek 2 system, *A. urinae* can be misidentified as a *Granulicatella* [4]. One isolate of *Granulicatella* from blood was identified and sequencing of the 16S rRNA gene revealed that this isolate was *A. urinae*. Twenty-seven isolates, 15 isolates from Lund and 12 isolates from the Malmö laboratory, that had been classified as streptococci but could be *A. urinae* (see methods) were subjected to 16S rDNA sequence analysis. Of these, three isolates were identified as *A. urinae*, whereas the remaining isolates were mainly of different streptococcal species (data not shown).

In total, 16 isolates of *A. urinae* were identified. In 15 patients *A. urinae* was identified from more than one blood culture. On most occasions (26 of 39 positive cultures), both the aerobic and anaerobic bottle grew *A. urinae*. In five patients *A. urinae* was only isolated from the anaerobic bottles. For 11 patients, *A. urinae* was the only organism isolated. In four patients, one bottle grew a coagulase-negative *staphylococcus* and in one patient the anaerobic bottle contained an anaerobic bacterium not further characterized.

This work represents the largest case series of bacteraemia with *A. urinae*. In a 6-year period we identified 16 cases in a population of one million inhabitants, which gives an incidence of approximately three cases per 1 000 000 inhabitants per year, in line with a recent report from the Netherlands [15] but significantly higher than figures reported from Denmark [11]. *A. urinae* is easily misclassified and despite our efforts to identify and reclassify historical isolates, we believe that several patients with *A. urinae* bacteraemia have been missed in our laboratories. *A. urinae* should always be suspected when a coccus growing in clusters has the appearance of a streptococcus on a plate, and ultimate species determination should rely on sequencing of the 16S rRNA-gene.

Lack of *A. urinae* in urinary cultures

For 13 patients, a urinary culture had been taken at the same time as the blood culture. In one patient the urine grew an aerococcus, whereas in four patients the urine grew α-haemolytic streptococci or *Enterococcus faecalis*. In five patients the urine was sterile and in an additional three patients other organisms were identified. We believe that *A. urinae* resided in the urine of at least some of the patients. The failure to detect *A. urinae* could be due to a combination of misidentification, suboptimal growth conditions for aerococci, and potential aerococci being regarded as non-significant.

Patient characteristics

Patients had a median age of 86 years (range 64–96) and 15 of 16 patients were male (Table 1). Nine patients had underlying urinary tract diseases such as prostate hyperplasia, or cancer

TABLE 1. Patient characteristics

Age, sex	Underlying urological disease	Underlying other disease	Initial symptoms	SIRS criteria	Organ dys-function	Treatment i.v.	Treatment p.o.	Diagnosis	Remarks
91, M	UC, BPH, RC	Colon cancer	Fe	3/4	0	PcG+Gm	0	IE	Received Ci for UTI. Spondylitis. Isolate resistant to Ci.
96, M	UC	IHD	Du	2/4	HT	Pt, Am, PcG	0	IE	Received Ci for UTI
87, M	–	Dementia	Scrotal pain	1/3	HT	Pt	Cef	Anal abscess	Isolate resistant to Ci
81, M	UC	CVI	Anxiety	2/4	Con	Ct	Am	Sepsis	
86, M	PC, urethral stricture	–	Fe, Du	2/4	Ren	Ct, PcG+Gm, Me	0	IE	Septic emboli to brain
82, M	PC, Bricker urostomy	–	Fe	–	0	Pt, PcG+Gm	PcV+Em	Sepsis	Iatrogenic at nephrostomy
80, M	UC	–	Fe	3/4	HT, HP	Le, PcG	0	Sepsis	Stop in UC
89, F	RC	–	Fe, angina pectoris	3/4	Resp	Pt	Cm	AMI	ST-elevation, TEE normal
74, M	Kidney stone	Dementia	Fe, loin pain	2/4	0	Ct	PcV+Ci	Pyelitis	Ureter concrement
72, M	–	CLL,COPD	Fe, back pain	0/1	0	Cf, PcG	Am	Spondylitis	Ru upon admission
87, M	–	MDS, CVI	Fe, shi	2/3	HP	Ip	Le	MDS	Ru upon admission
64, M	Penile cancer	IHD	Fe	2/3	0	Cf	Ci	Pyelitis	
76, M	BPH, bladder cancer	Dementia	Fe, shi	2/3	0	Ct	0	Sepsis	At UC change
92, M	–	–	Fe, shi, Du	1/4	0	Ct	Cm	Urinary retention	
78, M	PC, UC	–	Fe	4/4	Ren, Resp	Ct	0	PC	Succumbed after 48 h
88, M	Urethra cancer, UC	IHD, CVI	Confusion	2/2	Coa, Con, Ren	Ct	Ci+Cm	Urethral cancer	Received nephrostomy

The SIRS criteria are expressed as number of criteria met against the total number of criteria given in the medical records. Many patients received different antibiotics and the commas indicate changes of treatment whereas a plus indicates a combined treatment.
The following abbreviations were used: Am, amoxicillin; AMI, acute myocardial infarction; BPH, benign prostate hyperplasia; Cef, cefadroxil; Cf, cefuroxime; Ci, ciprofloxacin; CLL, chronic lymphocytic leukaemia; Cm, clindamycin; Coa, coagulation dysfunction; Con, confusion; COPD, chronic obstructive pulmonary disease; Ct, cefotaxime; CVI, cerebrovascular insult with sequelae; Du, dysuria; Em, erythromycin; F, female; Fe, fever; Gm, gentamicin; HP, hypoperfusion; HT, hypotension; IE, infectious endocarditis; IHD, ischaemic heart disease; Im, impenem; Le, levofloxacin; M, male; MDS, myelodysplastic syndrome; Me, meropenem; PC, prostate cancer; PcG, penicillin G; PcV, penicillin V; Pt, piperacillin-tazobactam; RC, renal cancer; Ren, renal dysfunction; Resp, respiratory dysfunction; Ru, residual urine; shi, shivers; TEE, transoesophageal echocardiography; UC, urinary catheter; UTI, urinary tract infection.

of the penis, prostate, urinary bladder or kidney, and six patients had a long-term urinary tract catheter. In total, 12 patients had either a diagnosed urinary tract disease or a urinary tract catheter. Six patients suffered from dementia or had sequelae after a previous stroke, three patients had ischaemic heart disease, and two patients had haematological malignancies (Table 1). Notably, none of the patients had diabetes.

Fever was a reported initial symptom in 12/16 patients and fever (>38°C) was recorded in 8/16 patients upon arrival at the hospital. In six patients initial symptoms such as dysuria, haematuria or loin tenderness indicated a urinary tract infection. Two patients had problems with their urinary tract catheter prior to disease onset and in two patients more than 1 L of residual urine was noted on admission. At presentation at the hospital, 12/16 patients fulfilled the criteria for the systemic inflammatory response syndrome (SIRS) [22] and data were partially missing for three of the remaining four patients. Three patients had hypotension upon admission, one of whom only fulfilled one SIRS criterion, and an additional six fulfilled different criteria for organ dysfunction (Table 1). Thus in total 12 patients fulfilled the criteria for sepsis and eight of these had severe sepsis. One patient fulfilled the criteria for severe sepsis but not for sepsis. The three patients that presented with hypotension responded to fluid therapy and therefore no patient was in septic shock upon admission.

Fifteen patients survived the hospital stay and one patient died. The non-survivor was a 78-year-old man, terminally ill with prostate cancer, who succumbed within 48 h from admittance. No patient received inotropic drugs or mechanical ventilation. Median duration of the hospital stay for surviving patients was 12 days (range 6–53 days).

In line with earlier studies, our results confirm that bacteraemia with *A. urinae* is a condition that mainly affects older males. The high frequency of underlying urinary tract disease and the high proportion of patients with symptoms from the urinary tract upon admission among our cases indicate that *A. urinae* in most cases resided in the urinary tract before dissemination to the bloodstream.

The majority of cases presented in this study fulfilled criteria for sepsis as they had SIRS caused by infection and we believe that the *A. urinae* bacteraemia was the cause of the symptoms in all of our cases. Importantly, *A. urinae* was the sole significant pathogen isolated in all our cases. Given the many co-morbidities and the high age of our patients we were surprised that only one fatality occurred.

Antibiotic susceptibility and antibiotic treatment

The MICs for relevant antibiotics were determined for 14 isolates using Etests (Table 2). As expected, low MIC values were recorded for penicillin, cefotaxime, clindamycin and vancomycin, whereas MICs for gentamicin ranged between 0.5 and 32 mg/L. Two isolates showed resistance to ciprofloxacin with MIC >32 mg/L.

TABLE 2. MIC in mg/L determined for 14 *A. urinae* isolates

	MIC_{50}	MIC_{90}
Penicillin G	<0.016	0.032
Cefotaxime	<0.016	0.125
Vancomycin	0.25	0.5
Gentamicin	2	16
Ciprofloxacin	0.125	32
Clindamycin	0.125	0.5

Fourteen patients received empirical antibacterial treatment with a beta-lactam antibiotic with a broad spectrum, such as a third-generation cephalosporin, piperacillin with tazobactam, or a carbapenem. Only two patients received a fluoroquinolone as empirical treatment; however, both these patients were later treated with a beta-lactam antibiotic. Intravenous treatment was given for a median time period of 7 days (range 0–40) whereas median total duration of treatment was 14 days (range 2–57). Oral treatment included ciprofloxacin or levofloxacin (n = 4), clindamycin (n = 3), a beta-lactam antibiotic (n = 3), or combinations of antibiotics (n = 3).

Aerococcus urinae is resistant to sulphamethoxazole and resistance to ciprofloxacin is observed in this study and has recently been reported [4,6]. Some patients with *A. urinae* bacteraemia will receive one of these drugs as empirical treatment for a suspected febrile UTI and development of fluoroquinolone resistance is thus troublesome.

Infectious endocarditis with *A. urinae*

Two of the patients were initially treated for a suspected bacteraemic urinary tract infection with ciprofloxacin but reappeared in the hospital during, or shortly after cessation of, the antibiotic treatment with fever. In both patients transoesophageal echocardiography (TEE) revealed vegetations on the mitralis or the aortic valves and renewed blood cultures demonstrated continued growth of *A. urinae*. The isolate from the patient with mitralis IE was resistant (MIC > 32 mg/L) to ciprofloxacin. A third patient had an aortic valve vegetation confirmed by TEE. In the three patients with IE, two had underlying urinary tract abnormalities, two had permanent urinary catheters, and two presented with symptoms suggesting urinary tract infection. One patient with IE was treated with penicillin alone and two patients were treated with penicillin in combination with gentamicin. One patient suffered a cerebral insult, presumably due to septic embolization, and one patient had clinical signs and a scintigraphic pattern consistent with a

spondylodiscitis (the patient had a pacemaker and thus MRI could not be performed). None of the patients with IE were subjected to valvular replacement surgery and none of the patients died. In previous reports on *A. urinae* IE, the case fatality was more than 50%, possibly indicating that more dramatic cases tend to be published as case reports [11,13–18]. Clinicians treating patients with *A. urinae* bacteraemia should always consider the possibility of IE, though this condition may not be as common as suggested by previous reports.

Of the 13 patients not diagnosed with IE, five patients were investigated with TEE and an additional three were investigated with transthoracic echocardiography. From reading the medical charts it appears that no evident case of IE was missed.

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Transparency Declaration

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